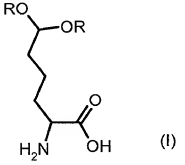
Title

Process for the preparation of allysine acetal

Background of the Invention

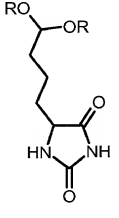
Field of the Invention

The present invention is directed towards the preparation of compounds of the general formula (I):



(I)

Such compounds are prepared in particular by means of an enzymatic process from hydantoins of the general formula (II):



(II)

Description of Related Art

Compounds of formula (I) are suitable intermediates for the preparation of pharmaceuticals described in US 5552397, WO 9738705 and in J. Med. Chem. 42, 305 (1999).

J. Med. Chem. 42, 305 (1999) mentions a synthesis route for the preparation of a structural unit - an α-amino-ε-caprolactam derivative - of the pharmaceutically active

compounds. However, that structural unit is obtained with the aid of expensive reagents in a process that is rather disadvantageous for a robust commercial process.

The preparation of compounds such as (I) from hydantoins such as (II) by means of *Arthrobacter* sp. is already known from JP 99206397. However, that document does not describe the advantageous racemisation.

The conversion of hydantoins by means of hydantoinases and specific carbamoylases is already known from DE19529211.1. The spontaneous chemical racemisation of hydantoins for the preparation of enantiomerically enriched amino acids is to be found in DE-P4137581.5-44.

Brief Description of the Invention

Accordingly, one object of the present invention is to provide an enzymatic process for the preparation of the desired compounds of formulae (I), that is a simpler, more efficient, and less costly process suitable for large-scale commercial or industrial applications.

Another object of the invention is to provide a process for providing an allysine acetal in high yield and with a high degree of optical purity, for instance L-allysine acetal in a yield of about 85% or more, preferably at least 90% and most preferably >95%, and with an optical purity of at least 90%, preferably at least 95%, and most preferably >99%ee.

Yet another object of the invention is to provide a process that uses a total cell catalyst comprising a cell that has a gene encoding a hydantoin racemase, a hydantoinase and an L- or D-specific carbamoylase. For instance, a total cell catalyst comprising an L-specific carbamoylase.

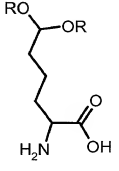
Products and compositions comprising compounds of general formulae (I) and having a high degree of optical purity are advantageously used as intermediates for producing

pharmacologically active products, or directly in pharmacological products.

Other objects of the invention are described or may be inferred from the following detailed description.

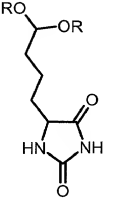
Detailed Description of the Invention

The process according to the present invention comprises the preparation of an allysine acetal of the general formula (I):



(I)

from a starting material comprising a hydantoin of the general formula (II):



(II)

In both formulae (I) and (II) above: R represents (C_1-C_8)-alkyl, (C_2-C_4)-alkylenyl, preferably ethylenyl, (C_6-C_{18})-aryl, (C_7-C_{19})-aralkyl, or (C_1-C_8)-acyl.

(C_1-C_8)-alkyl is a saturated hydrocarbon radical, such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl,

sec-butyl, *tert*-butyl, pentyl, hexyl, heptyl or octyl, including all their isomers.

A (C_2-C_8)-alkylenyl radical refers to an unsaturated hydrocarbon radical having from 2 to 8 carbon atoms containing at least one double bond, such as, for example, ethylenyl, propylenyl, etc. as well as isomers of these radicals.

A (C_6-C_{18})-aryl radical is to be understood as being an aromatic radical having from 6 to 18 carbon atoms. It includes in particular groups such as phenyl, naphthyl, anthryl, phenanthryl, biphenyl radicals as well as isomers of these groups.

A (C_7-C_{19})-aralkyl radical is a (C_6-C_{18})-aryl radical bonded to the molecule *via* a (C_1-C_8)-alkyl radical.

A (C_1-C_8)-acyl radical denotes a (C_1-C_8)-alkyl radical that is bonded to the molecule *via* a C=O function.

The structures of the compounds shown in Formulas (I) and (II) above relate to both optical isomers.

Compounds of formula (II) are subjected to a reaction with at least one hydantoinase and at least one D- or L-specific carbamoylase, as well as to a spontaneous and/or enzyme-catalysed *in situ* racemisation. The enzymes involved may be used in free form, in immobilised form, as cell fractions or extracts or in a form enclosed in a cell. The desired compounds such as those of formula (I) are more easily obtained in advantageous yields and purities in a manner that is surprising for a large-scale process.

The reaction sequence according to the invention has hitherto not been applied in the prior art to the present compounds. It is therefore to be regarded as surprising that the labile acetal protecting group is stable under the reaction conditions and allysine acetal having an optical purity >99%ee can be generated in a very high yield from 100% of the hydantoin in an overall yield >85%.

As has been mentioned, the process according to the invention may be carried out partially enzymatically or completely enzymatically. In addition to the use of the free enzymes in a reaction batch, special preference is given, however, to a process in which there is used a so-called whole cell catalyst from a cell that has a cloned gene coding for a hydantoin racemase, a hydantoinase and an L- or D-specific carbamoylase. Such organisms are known in principle from US 60/157427 (SEQ ID NO: 4/hydantoinase, SEQ ID NO:5/hydantoin racemase, SEQ ID NO:6/carbamoylase) or US 09/407062 (SEQ ID NO:1/hydantoinase, SEQ ID NO: 2/hydantoin racemase, SEQ ID NO: 3/carbamoylase). Accordingly, the disclosure of those specifications is regarded as being included herein, especially the disclosure of the relevant amino acid sequences in the sequence listings. That is the case especially for US 60/157427.

The use of a total cell catalyst having an L-specific carbamoylase is most particularly advantageous. It is thus possible to obtain the desired optical antipode of allysine acetal for the preparation of pharmaceutically active substances.

In principle, the total cell catalyst may be any suitable expression system that comes into consideration for that purpose to those skilled in the art. Special preference is given, however, to the use of a recombinant bacterium, preferably *E. coli*, for this purpose. Advantageous *E. coli* strains include: JM109, NM 522, JM105, RR1, DH5α, TOP 10^{-} or HB101.

In order to carry out the invention, the following procedure is generally followed:

the substrate (II) is brought into contact with the enzymes in a suitable solvent, preferably water, at an optimum pH value for hydantoinase and carbamoylase of approximately from 5.5 to 8.5, preferably from 6.5 to 8, and at an optimum temperature for the enzyme activity of approximately from 20°C to 40°C, preferably from 25°C to 35°C.

Advantageously metal salts, such as $CoCl_2$ or $MgCl_2$, $MnCl_2$, etc., that have a positive effect on the enzyme activities may be added.

During cleavage of the hydantoins into the optically enriched amino acids, the hydantoins (or N-carbamoyl amino acids produced) racemise spontaneously. However, in order to accelerate that reaction, the hydantoin that remains can be racemised enzymatically *in situ* and is thus again available for cleavage into the amino acid. Enzymatic racemisation that proceeds simultaneously with the conversion of the hydantoin to the amino acid is preferred for reasons of simplicity, expediency or efficiency. All of the hydantoin can thus be converted into the amino acid in one step.

This reaction may be performed by using separate enzymes [that are available separately, which]. Such enzymes may be in free or immobilised form, be contained within a cell fraction or extract or be enclosed inside of a microorganism (US 60/157427).

The process according to the invention may be carried out in sequential reaction batches or continuously in a so-called enzyme-membrane reactor (Wandrey *et al.* in Jahrbuch 1998, Verfahrenstechnik und Chemieingenieurwesen, VDI p. 151 ff; Kragl *et al.* Angew. Chem. 1996, 6, 684 f).

Acetals produced by the processes of the present invention may be further purified by conventional methods known in the art, for instance, by separation of acetal from contaminants by centrifugation, extraction, or filtration and by recovering acetals by crystalization.

In a further embodiment, the invention relates to the use of the acetals prepared according to the invention as intermediates for synthesis or preparation of active ingredients, especially pharmaceutical compounds having biological activity.

Acetals prepared according to the processes of the present invention may also be directly incorporated into pharmaceutical compositions in combination with other pharmaceutically acceptable ingredients, excipients or carriers.

The following example further elaborates on one embodiment of the present invention. The process of the present invention may be practiced in other ways than as specifically described below and therefore is not limited by this example.

Example:

30 g (wet weight) of *E. coli* cells JM109 (pOM22, pOM21) (US 60/157427) were mixed together with 100 mM DL-allysine hydantoin and 1mM $CoCl_2$ at pH 7.8 in 1 litre of water. The reaction mixture was then left for 4 hours at 37°C. The cells were then centrifuged off (45 min, 8000 rpm, 4°C, Beckman Coulter JA-10 rotor) and the supernatant was analysed by means of HPLC. After 4 hours, a yield of L-allysine acetal of >85% having an optical purity of >99%ee was obtained.

Modifications and other embodiments

Various modifications and variations of the described processes, products and compositions as well as the concept of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed is not intended to be limited to such specific embodiments. Various modifications of the described modes for carrying out the invention which are obvious to those skilled in the biological, chemical, chemical engineering, medical or pharmacological arts or related fields are intended to be within the scope of the following claims.

Incorporation by Reference

Each document, patent application or patent publication cited by or referred to in this disclosure is incorporated by reference in its entirety. Any patent document to which this application claims priority is also incorporated by reference in its entirety. Specific incorporation by reference is made to DE 100 37 115.9, filed July 28, 2000.